

Anthelmintic Efficacy of Soluble Formulation of A Benzimidazole Carbamate A Broad Spectrum Anthelmintic

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Abstract: Methyl-[5-[(4-(2-pyridinyl)-1-piperazinyl)-carbonyl]-1H-benzimidazol-2-yl]-carbamate (CDRI Compound 81/470) has consistently exhibited broad-spectrum anthelmintic efficacy both against adult and larval stages of various helminth parasites of experimental and domestic animals, by oral and parenteral routes. The compound incorporated in transdermal tapes has also yielded anthelmintic action.

With a view to enhance absorption and consequently the efficacy, a soluble formulation of the compound has been developed and its efficacy was evaluated against various experimental helminth parasites of rodents (*Ancylostoma ceylanicum* (adult), *Nippostrongylus brasiliensis* (adult and L₄), *Syphacia obvelata* (adult)) and poultry (*Ascaridia galli*). This appears to be the only benzimidazole which could be solubilized.

While the soluble formulation has been found to have comparable efficacy to the powder form against intestinal worms, the efficacy was substantially increased against systemic parasites (*N. brasiliensis* L₄ stage). The enhanced efficacy by oral route is very well correlated with high absorption of drug from soluble formulation which has been demonstrated by the pharmacokinetic studies reported by Nagaraja *et al.* (1995).

The results of the present study suggest that the soluble formulation may find wider application in respect to easy administration through drinking water in large herds, flocks and also against tissue dwelling parasites specially filarids.

Key words: Anthelmintic efficacy, Benzimidazole carbamate, soluble formulation, *Ancylostoma ceylanicum*, *Nippostrongylus brasiliensis*, *Syphacia obvelata*, *Ascaridia galli*.

INTRODUCTION

Methyl-[5-[(4-(2-pyridinyl)-1-piperazinyl)-carbonyl]-1-H-benzimidazol-2-yl]- carbamate (CDRI Compound 81/470) has consistently exhibited broad-spectrum anthelmintic efficacy both against adult and larval stages of various helminth parasites of experimental and domestic animals by oral and parenteral routes (Katiyar *et al.*, 1984, 1987, 1988). The compound incorporated in transdermal tape has also shown excellent action (Jain *et al.*, 1991; Gupta *et al.*, 1992).

With a view to enhance bioavailability and consequently the efficacy, a soluble formulation of the compound has been developed (Jain *et al.*, 1994) and evaluated against various helminth parasites of rodents and poultry.

MATERIALS AND METHODS

Test Parasites, Animals and Treatment:

Compound 81/470 (soluble form) was tested against adult *Ancylostoma ceylanicum* in hamsters, adult and larvae of *Nippostrongylus brasiliensis* in rats, adult *Syphacia obvelata* in mice and *Ascaridia galli* in fowl. Simultaneous comparison was made with powder form of the compound.

Laboratory bred adult male hamsters (50–60 g), newly weaned young male rats of UF strain (35–40 g), male albino swiss mice (18–20 g) and naturally infected birds (fowl) served as experimental hosts. Rodents were housed in plastic cages in a climatically controlled animal house (25–28°C) and had free access to standard rodent food pellets (Lipton India Ltd.) and water. *A. galli* infected fowls (ascertained by stool examination) were maintained in individual cage and were provided with poultry feed and water. The animals were killed under deep ether anaesthesia.

Preparation of soluble formulation:

100mg/ml solution of 81/470 was prepared by dissolving 1 g of compound in 5 ml lactic acid at room temperature. To the resultant solution 5 ml of distilled water was added, pH of the solution was adjusted to 3.0 by adding, in portion, solid sodium bicarbonate. The solution was filtered through a sintered funnel to remove any undissolved or suspended impurities.

Anthelmintic assay:

The powder and soluble forms were simultaneously evaluated.

1. *Ancylostoma ceylanicum* (hook worm):

The therapeutic efficacy was assessed by the procedure described earlier by Ray *et al.* (1978) and Katiyar *et al.* (1984, 1987). Hamsters were orally infected with 60 ± 5 infective larvae (L_3) of *A. ceylanicum*. The animals found positive by ovoscopic examination on day 17-post-infection (p.i.) were used in therapeutic trials. The positive animals of one infected

batch were randomly allocated for control and for treatment with both the formulations of compound. The administration of both the formulations, at 6.25, 3.12 & 1.56 mg/kg \times 1 dose levels, was done on any day between day 17 and 20 p. i. The efficacy was expressed in terms of absolute clearance of parasites from the host and percent worm reduction. The percent worm reduction in the treated group compared to untreated control group was obtained by the formula $(N-n) \times 100 \div N$ (where N and n stand for average numbers of worms in untreated control group and treated group respectively).

2. *Nippostrongylus brasiliensis* (Trichostrongylid):

Adulticidal

The rats were infected with 500 L₃ subcutaneously (Misra *et al.* 1981). The therapeutic trials at 100, 50, 25 and 12.5 mg/kg \times 3 days dosage regimen were initiated on day 9 post infection and continued for three consecutive days. The efficacy assessment was done in the manner described for *A. ceylanicum* (adulticidal).

Larvicidal

The larvicidal action was evaluated against L₄ stage in lungs as described by Katiyar *et al.* (1987) and Gupta *et al.* (1992). Since the abode of L₄ is lung, the formulation of the compound were tested also by intramuscular route. The two formulations were administered on day two of larval inoculation at the dose levels of 20 mg/kg \times 1 by i. m. and p. o. routes and treated animals were sacrificed on day 9 p. i.. The adult worms were enumerated (Katiyar *et al.*, 1987) and efficacy assessment was made as described above.

3. *Syphacia obvelata* (Oxyurid):

Adult mice of either sex, 9–10 months old (25–30 g) harbouring a natural infection of *S. obvelata* were treated with both the formulations at different dose levels (Table 1). The mice freed from the worms, as observed on sacrifice, formed the basis of drug efficacy (Katiyar *et al.*, 1982).

4. *Ascaridia galli* (Ascarid):

The adult fowls positive for round worms, were procured from local poultry farm. Both the formulations were assessed by oral route at three dose levels (20, 10 and 5 mg/kg \times 1). After treatment, the stool voided each day was examined for expelled worms. When the worm expulsion ceased the birds were sacrificed and whole of the intestine was slit open and carefully observed for worms to verify drug's efficacy. Untreated control animals were similarly examined for the presence of worms in their stool and in the intestine on sacrifice (Katiyar *et al.*, 1984). Complete cure was the criterion of efficacy.

RESULTS

Efficacy profile

The efficacy of powder and soluble form of the compound 81/470 is presented in Table 1 and Table 2.

Table 1. Anthelmintic efficacy of powder and soluble form of Compound 81-470 against enteric helminths

Parasite (host)	Dose mg/kg	Powder form			Soluble form			
		Animals cured	Percent cure	Worms recovered	Percent worm reduction	Animals cured	Percent cure	Worms recovered
		Animals treated (replicate)		Mean \pm SD		Animals treated (replicate)		Mean \pm SD
<i>A. ceylanicum</i> (Hamster)	6.25 \times 1	23/23 (7)	100.0	0 \pm 0	100	23/23 (7)	100.0	0.0
	3.12 \times 1	18/24 (7)	75.0	0.875 \pm 2.346	96.71	18/24 (7)	75.0	1 \pm 2
	1.56 \times 1	15/28 (7)	53.6	3.57 \pm 4.90	86.59	11/28 (7)	39.0	3.86 \pm 4.38
(Control)	0 \times 1	0/22	—	26.636 \pm 9.016	—	—	—	—
<i>N. brasiliensis</i> (Rat)	100 \times 3	13/13 (4)	73.3	0 \pm 0	100	13/13 (4)	100.0	0.0
	50 \times 3	11/15 (4)	73.3	0.87 \pm 1.81	99.37	14/20 (4)	70.0	3.70 \pm 6.46
	25 \times 3	6/15 (3)	40.0	7.07 \pm 10.22	94.91	5/10 (3)	50.0	9.30 \pm 13.69
	12.5 \times 3	0/8 (3)	0.0	62.22 \pm 36.82	55.22	0/11 (3)	0.0	71.00 \pm 33.48
(Control)	0 \times 3	0/17	—	138.94 \pm 46.28	—	—	—	—
<i>S. obvelata</i> (Mouse)	100 \times 3	12/12 (4)	91.6	—	—	12/12 (4)	100.0	—
	50 \times 3	14/18 (5)	77.0	—	—	14/16 (5)	87.5	—
	25 \times 3	8/13 (3)	61.53	—	—	6/9 (3)	66.7	—
<i>A. galli</i> (Fowl)	20 \times 1	6/6 (2)	100.0	—	—	6/6 (2)	100.0	—
	10 \times 1	6/6 (2)	100.0	—	—	6/6 (2)	100.0	—
	5 \times 1	3/5 (2)	60.0	—	—	4/6 (2)	66.7	—

Significance: Powder form vs Soluble form - NS ($P > 0.05$)

Table 2. Efficacy of powder and soluble form of Compound 81-470 against tissue dwelling *L*₄ stage of *N. brasiliensis*

Group	Dose mg/kg	Powder form (a)			Soluble Form (b)				
		Rats cured Rats treated (replicate)	Percent cure	Worm recovered Mean \pm SD	Present worm reduction	Rats cured Rats treated (replicate)	Percent cure	Worm recovered Mean \pm SD	Present worm reduction
1	20 \times 1 (im)	0/12 (2)	0.0	50 \pm 19.76	64.3	0/12 (2)	0.0	42.50 \pm 24.64	69.6
2	20 \times 1 (p.o)	0/11 (2)	0.0	28.27 \pm 20.59	79.8	0/11 (2)	0.0	11.18 \pm 8.59	92.0
3	0 \times 1 (Control)	0/10	—	140.10 \pm 31.81					

Significance:		p.o	
Group a vs b		im	P < 0.05
		NS	
		Powder	Soluble
	1 vs 2	P < 0.05	P < 0.01
	1 vs 3	P < 0.01	P < 0.01
	2 vs 3	P < 0.01	P < 0.01

A. ceylanicum (Adulticidal activity):

Both powder and soluble formulations at single oral dose of 6.25 mg/kg resulted in complete elimination of parasites. Lower doses yielded dose dependent efficacy (Table 1). There is no significant difference in activity ($p > 0.05$) between the two formulations at each dose level.

N. brasiliensis (Adulticidal activity):

Different log doses of both the formulations were used. 100 mg/kg dose given for three consecutive days cured all the rats. At lower dose of 25 mg/kg \times 3, more or less similar activity (93–95 %) was recorded with both the formulations. Reduction in dose had proportionate activity.

N. brasiliensis (Larvicidal activity):

The activity of soluble preparation against L_4 stage was significantly enhanced ($p < 0.05$). The powder form at 20 mg/kg \times 1 dose level exhibited 64.3 % and 78.8 % worm reduction by i. m. and oral routes respectively while the values with soluble formulation were 69.6 % and 92.0 % (Table 2). By oral route the efficacy of soluble formulation was significantly higher ($p < 0.05$) than the powder form, whereas by i. m. route there was no marked difference in activity (NS).

S. obvelata (Adulticidal activity):

Both the formulations showed similar activity at 100, 50 and 25 mg/kg \times 3 dosage regimen (Table 1).

A. galli (Adulticidal activity):

All the fowls were successfully cured when treated at 20 and 10 mg/kg \times 1 dose levels of the two formulations. Proportionally reduced efficacy was recorded at 5 mg/kg \times 1 dose level (Table 1).

DISCUSSIONS

Benzimidazole constitute an important class of anthelmintics, however, they are notoriously insoluble in water (Toeco *et al.*, 1965; Sharma and Srivastava, 1988) which limits their use against systemic parasites. In order to increase bioavailability and consequently the efficacy, efforts were directed to make a soluble formulation of the compound while retaining anthelmintic activity. It appears to be the first example amongst the benzimidazole derivatives which could be successfully solubilised without loss of biological activity.

Amongst the four enteric helminths studied, only *N. brasiliensis* has systemic migration and L_4 stages inhabit lungs. Therefore the anthelmintic efficacy of both the formulations was studied only against L_4 stage of *N. brasiliensis*. The larvae (L_3 of *N. brasiliensis* after inoculation in rats reach the lungs, lymph and blood vascular systems 30–36 hr after inoculation and moult to L_4 . By 65 hr the L_4 stages (35% of original L_3 dose) are carried to the lumen of the small intestine where they mature. Eggs appear in the faeces by day 6 following infection (Haley *et al.*, 1962; Ogilvie and Jones, 1971). To study the action against L_4 stage,

the compound was administered after 30–36 hr p. i. (Katiyar *et al.*, 1987; Gupta *et al.*, 1992) and larvicidal efficacy, which otherwise was indirect, assessed on day 9 p. i..

While the soluble formulation has been found to have comparable efficacy to the powder form against intestinal worms, the efficacy was substantially increased against systemic parasites (*N. brasiliensis* L₄ stage).

Against L₄ stage both the formulations of the compound 81/470 given orally yielded better efficacy than by parenteral route. The findings are in confirmation of our previous observations (Katiyar *et al.*, 1987). Enhanced efficacy by oral route could be due to better absorption of the drug from soluble formulation. It has already been demonstrated by Nagaraja *et al.* (1995) through pharmacokinetic studies of suspension and soluble formulation that later is 2.14 times better absorbed than suspension form.

The results of the present study suggest that the soluble formulation may find wider application in respect to easy administration through drinking water in large herds/ flocks and against tissue dwelling parasites especially filarids.

In view of the recent findings, the compound 81/470, having easy chemical synthesis and better activity profile in soluble form against tissue dwelling parasites, appears to be a strong candidate anthelmintic for veterinary use.

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